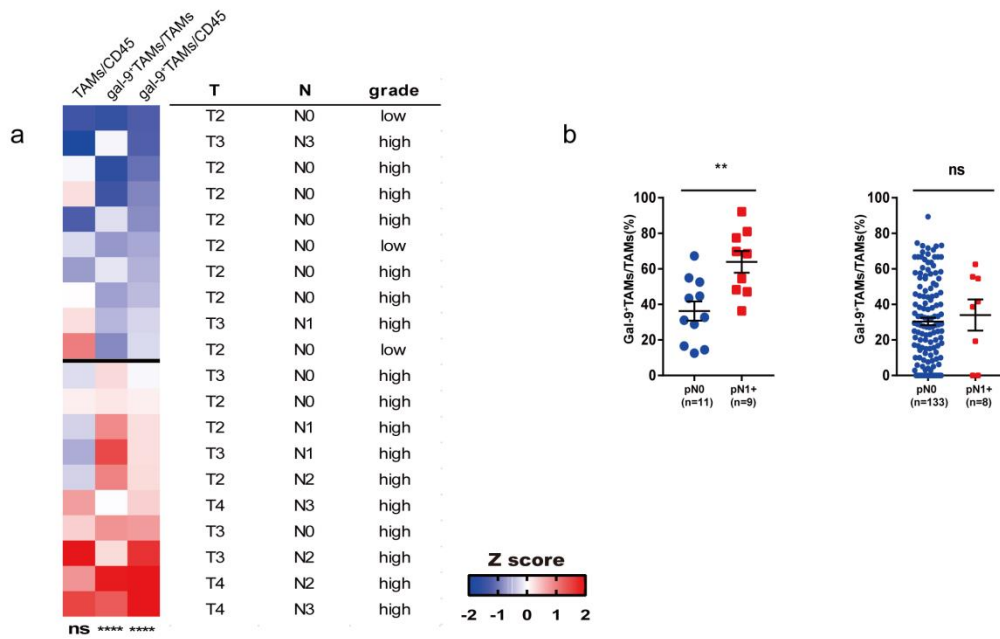


Supplementary Figure 1



Supplementary Figure 1. Clinical characteristics of the patient cohort from the Shanghai Cancer Center

(a) Clinical characteristics of the patients whose tumor specimens are used in this study and are segregated by the median of gal-9⁺TAMs/CD45⁺ cells by FCM. Data for each column are normalized as the Z score. (b) Percentage of gal-9⁺TAMs among different node stages observed by FCM (left) and IHC (right).

Supplementary Table 1. Baseline demographics and clinical characteristics of the Zhongshan Hospital cohort

Characteristics	Patients (n=141)	
	n	%
Age at surgery (years)		
≤62	72	51.1
>62	69	48.9
Sex		
Male	117	83.0
Female	24	17.0
Tumor size (cm)		
≤3.5	73	51.8
>3.5	68	48.2
pT stage		
T2	90	63.8
T3	30	21.3
T4	21	14.9
pN stage		
pN0	133	94.3
pN+	8	5.7
AJCC stage		
II	87	61.7
III	46	32.6
IV	8	5.7
Grade		
Low grade	24	17.0
High grade	117	83.0
LVI		
Absent	52	36.9
Present	89	63.1
Adjuvant chemotherapy		
Applied	69	48.9
Not applied	72	51.1
Events		
Death	75	53.2
Recurrence	68	48.2

Abbreviations: AJCC: American Joint Committee on cancer; LVI: Lymphovascular invasion

Supplementary Table 2. Correlations between TAMs, Gal-9⁺TAMs (%), Gal-9⁺TAMs (%) and clinical characteristics in the Zhongshan cohort

Factor	TAMs (CD68)		P*	Gal-9 ⁺ TAMs (%)		P*	Gal-9 ⁺ TAMs (%)		P*
	Low (<62 cells/HPF)	High (≥62 cells/HPF)		Low (<25%/HPF)	High (≥25%/HPF)		Low (<62%/HPF)	High (≥62%/HPF)	
Age at surgery (years)			0.266			0.661			0.362
≤62	37 (26.2%)	35 (24.8%)		35 (24.8%)	37 (26.2%)		31 (22.0%)	41 (29.1%)	
>62	29 (20.6%)	40 (28.4%)		31 (22.0%)	38 (27.0%)		35 (24.8%)	34 (24.1%)	
Sex			0.428			0.579			0.916
Male	53 (37.6%)	64 (45.4%)		56 (39.7%)	61 (43.3%)		55 (39.0%)	62 (44.0%)	
Female	13 (9.2%)	11 (7.8%)		10 (7.1%)	14 (9.9%)		11 (7.8%)	13 (9.2%)	
Tumor size (cm)			0.779			0.954			0.954
≤3.5	35 (24.8%)	38 (27.0%)		34 (24.1%)	39 (27.7%)		34 (24.1%)	39 (27.7%)	
>3.5	31 (22.0%)	37 (26.2%)		32 (22.7%)	36 (25.5%)		32 (22.7%)	36 (25.5%)	
pT stage			0.880			0.028			0.049
T2	42 (29.8%)	48 (34.0%)		49 (34.8%)	41 (29.1%)		38 (27.0%)	52 (36.9%)	
T3	15 (10.6%)	15 (10.6%)		12 (8.5%)	18 (12.8%)		13 (9.2%)	17 (12.1%)	
T4	9 (6.4%)	12 (8.5%)		5 (3.5%)	16 (11.3%)		15 (10.6%)	6 (4.3%)	
pN stage			0.018			0.858			0.582
pN0	66 (46.8%)	67 (47.5%)		63 (44.7%)	70 (49.6%)		61 (43.3%)	72 (51.1%)	
pN+	0 (0.0%)	8 (5.7%)		3 (2.1%)	5 (3.5%)		5 (3.5%)	3 (2.1%)	
AJCC stage			0.022			0.092			0.375
II	42 (29.8%)	45 (31.9%)		47 (33.3%)	40 (28.4%)		37 (26.2%)	50 (35.5%)	
III	24 (17.0%)	22 (15.6%)		16 (11.3%)	30 (21.3%)		24 (17.0%)	22 (15.6%)	
IV	0 (0.0%)	8 (5.7%)		3 (2.1%)	5 (3.5%)		5 (3.5%)	3 (2.1%)	
Grade			0.032			0.010			0.428

Low grade	16 (11.3%)	8 (5.7%)		17 (12.1%)	7 (5.0%)		13 (9.2%)	11 (7.8%)	
High grade	50 (35.5%)	67 (47.5%)		49 (34.8%)	68 (48.2%)		53 (37.6%)	64 (45.4%)	
LVI			0.020			0.352			0.103
Absent	31 (22.0%)	21 (14.9%)		27 (19.1%)	25 (17.7%)		29 (20.6%)	23 (16.3%)	
Present	35 (24.8%)	54 (38.3%)		39 (27.7%)	50 (35.5%)		37 (26.2%)	52 (36.9%)	
Adjuvant chemotherapy									
Applied	30 (21.3%)	39 (27.7%)	0.438	35 (24.8%)	37 (26.2%)	0.661	33 (23.4%)	36 (25.5%)	0.813
Not applied	36 (25.5%)	36 (25.5%)		31 (22.0%)	38 (27.0%)		33 (23.4%)	39 (27.7%)	

*Fisher's exact test is used when table data do not meet the requirement of the Chi-square test.

p-value < 0.05 marked in bold font means statistical significance.

Abbreviations: AJCC: American Joint Committee on cancer; LVI: Lymphovascular invasion

Supplementary Table 3. Univariate and multivariate Cox regression analyses of clinico-pathological features associated with overall survival and recurrence-free survival of MIBC patients

	OS (n=141)		RFS (n=114)	
	HR (95% confidence interval)	P	HR (95% confidence interval)	P
Univariate				
pT stage (pT3+pT4 vs. pT2)	1.497 (0.947-2.367)	0.084	1.226 (0.640-2.349)	0.538
pN stage (pN+ vs. pN0)	2.273 (0.981-5.266)	0.055		
Grade (high vs. low)	2.661 (1.274-5.557)	0.009	4.125 (1.480-11.491)	0.007
LVI (absent vs. present)	1.633 (1.002-2.659)	0.049	1.313 (0.741-2.326)	0.350
ACT (applied vs. not applied)	0.786 (0.494-1.250)	0.309	0.554 (0.308-0.997)	0.049
TAMs (high vs. low)	1.361 (0.856-2.166)	0.193	1.346 (0.771-2.348)	0.296
Gal-9 ⁺ TAMs (%) (high vs. low)	2.782 (1.663-4.654)	<0.001	2.171 (1.216-3.874)	0.009
Gal-9 ⁻ TAMs (%) (high vs. low)	0.720 (0.456-1.137)	0.159	1.087 (0.619-1.907)	0.771
Multivariate				
pT stage (pT3+pT4 vs. pT2)	1.334 (0.772-2.306)	0.302	1.616 (0.746-3.499)	0.223
pN stage (pN+ vs. pN0)	1.825 (0.738-4.515)	0.193		
Grade (high vs. low)	1.909 (0.901-4.047)	0.092	3.521 (1.251-9.913)	0.017
LVI (absent vs. present)	1.341 (0.793-2.269)	0.273	1.000 (0.542-1.847)	0.999
ACT (applied vs. not applied)	0.548 (0.321-0.935)	0.027	0.386 (0.194-0.770)	0.007
TAMs (high vs. low)	1.229 (0.758-1.992)	0.402	1.404 (0.783-2.517)	0.255
Gal-9 ⁺ TAMs (%) (high vs. low)	2.917 (1.581-5.383)	0.001	2.450 (1.237-4.853)	0.010
Gal-9 ⁻ TAMs (%) (high vs. low)	1.057 (0.619-1.805)	0.839	1.618 (0.828-3.161)	0.159

Abbreviations: HR, Hazard ratio; LVI: Lymphovascular invasion; ACT: Adjuvant chemotherapy;

TAMs: tumor associated macrophages

p-value < 0.05 marked in bold font means statistical significance.

Supplementary Table 4. IHC antibodies

Markers	Main target	sources	Species	Dilution	Antigen Retrieval	Cellular localization
Single-staining						
CD8	T cells	Abcam, ab17147	Mouse monoclonal	1:100	Citrate buffer (pH 6.0) microwave 20 min	Membranous
CD19	B cells	Abcam, ab31947	Mouse monoclonal	1:400	Citrate buffer (pH 6.0) microwave 20 min	Membranous
FoxP3	Treg cells	Abcam, ab22510	Mouse monoclonal	1:100	Citrate buffer (pH 6.0) microwave 20 min	Nuclear
Tryptase	Mast cells	Abcam, ab2378	Mouse monoclonal	1:10000	Citrate buffer (pH 6.0) microwave 20 min	Cytoplasmic
CD56	NK cells	Dako, clone M7304	mouse polyclonal	1:50	Citrate buffer (pH 6.0) microwave 20 min	Cytoplasmic
CD66b	Neutrophils	Abcam, ab197678	Rabbit monoclonal	1:1000	Citrate buffer (pH 6.0) microwave 20 min	Membranous
Double-staining						
CD11c	DC	Abcam, ad52632	Rabbit monoclonal	1:200	Citrate buffer (pH 6.0) microwave 20 min	Membranous
HLA-DR		Abcam, ab20181	Mouse monoclonal	1:200		
CD4	Th1 cells	Abcam, ab67001	Mouse monoclonal	1:50	Citrate buffer (pH 6.0) microwave 20 min	Membranous
T-bet		Abcam, ab150440	Rabbit monoclonal	1:500		
CD4	Th2 cells	Abcam, ab67001	Mouse monoclonal	1:50	Citrate buffer (pH 6.0) microwave 20 min	Membranous
GATA-3		Abcam, ab186371	Rabbit monoclonal	1:100		
CD68	Gal-9 ⁺ macro	Dako, clone KP1	Mouse monoclonal	original dilution	Citrate buffer (pH 6.0) microwave 20 min	Cytoplasmic
Galectin-9	phages	Abcam, ab69630	Rabbit monoclonal	1:500		

Supplementary Table 5. Specific markers for FCM

Antibodies	Source	Fluorochrome	Identifier
Fixable Viability Stain 510	BD Pharmingen	BV510	Cat# 564406
anti-CD8 (RPA-T8)	BD Pharmingen	BB515	Cat# 564526
anti-CD4 (RPA-T4)	BD Pharmingen	BV421	Cat# 562424
anti-CD68 (Y1/82A)	BD Pharmingen	AF647	Cat# 562111
anti-CD25 (M-A251)	BD Pharmingen	FITC	Cat# 555431
anti-CD56 (B159)	BD Pharmingen	BB515	Cat# 564488
anti-EpCAM (EBA-1)	BD Pharmingen	BV421	Cat# 563180
anti-FOXP3 (236A/E7)	BD Pharmingen	AF647	Cat# 561184
anti-GZMB (GB11)	BD Pharmingen	AF647	Cat# 560212
anti-PRF1 (δG9)	BD Pharmingen	PE	Cat# 556437
anti-PD-1 (EH12.1)	BD Pharmingen	AF647	Cat# 560838
anti-TIM-3 (7D3)	BD Pharmingen	PE	Cat# 563422
anti-Galectin-9 (9M1-3)	BD Pharmingen	PE	Cat# 565890
anti-IFNγ	BD Pharmingen	PE-CY7	Cat# 557643
anti-IL10 (JES3-9D7)	BD Pharmingen	BV650	Cat# 564051
anti-Annexin V	BD Pharmingen	PE	Cat# 561012
anti-CD45 (2D1)	BD Pharmingen	APC-CY7	Cat# 557833
anti-CD11b (ICRF44)	BD Pharmingen	FITC	Cat# 562793
anti-LAG3 (11C3C65)	Biolegend	BV785	Cat# 369322
anti-TGFβ1 (TW4-2F8)	Biolegend	PE-CY7	Cat# 349610
anti-Ki67 (Ki-67)	Biolegend	AF700	Cat# 350530
anti-CD3 (HIT3a)	Biolegend	Percp 5.5	Cat# 300328
anti-CTLA4 (BNI3)	Biolegend	BV605	Cat# 369610
anti-TIGIT (741182)	R&D	APC-R700	Cat# FAB7898N

Supplementary Table 6. Spearman's correlation between different cell types and gal-9⁺TAMs (%) or TAMs

Cell types	Gal-9 ⁺ TAMs (%)		TAMs	
	Spearman's rho	P value	Spearman's rho	P value
CD8 ⁺ T	-0.192	0.022	0.156	0.064
DC	-0.173	0.041	0.037	0.659
Treg	0.310	<0.001	0.309	<0.001
Mast	0.246	0.003	0.106	0.213
B	0.161	0.057	0.254	0.002
NK	-0.007	0.930	0.099	0.244
N	0.159	0.060	0.361	<0.001
Th1	-0.014	0.872	0.092	0.280
Th2	0.140	0.098	0.165	0.050
TAMs	0.315	<0.001	—	—

p-value < 0.05 marked in bold font means statistical significance.

Abbreviations: TAMs: tumor associated macrophages